



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

August 18, 2003

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 1677-193
DP Barcode: D290090

From: Chris Jiang, Chemist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Through: Emily Mitchell, Team Leader *Emily Mitchell 9/5/03*
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To: Marshall Swindell, PM 33 / Portia Jenkins
Regulatory Management Branch I
Antimicrobials Division (7510C)

Applicant: Ecolab, Inc.
840 Sibley Memorial Highway
St. Paul, MN 55118

Formulation From Label:

<u>Active Ingredient(s)</u>	<u>% by wt</u>
Peroxyacetic acid	15.2
Hydrogen peroxide	11.2
<u>Inert Ingredient(s)</u>	<u>73.6</u>
Total	100.0

I BACKGROUND

The product, AdvaCare 120 Sanitizer/Sour (EPA Reg. No. 1677-193), is an EPA-approved laundry additive for sanitizing laundry during commercial-industrial-institutional laundry operations. The product label lists the active ingredients as peroxyacetic acid and hydrogen peroxide. The applicant requested an amendment to their label to reflect effectiveness of the product, currently approved for use at 90°F, at a higher temperature of 140°F. The studies were conducted at AppTec ATS, located at 2540 Executive Drive, St. Paul, MN 55120.

This data package contained EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-35 (Data Matrix), two studies (MRID Nos. 459159-01 and 459159-02), Statements of No Data Confidentiality Claims for both studies, and a proposed label. The only differences between the proposed label and the last accepted label are that "AdvaCare 120 Sanitizer/Sour" has replaced "AdvaCare" and the range of "90 °F to 140 °F" has replaced the temperature of "90 °F."

II USE DIRECTIONS

The product is designed to be used as a laundry additive for sanitizing laundry during commercial-industrial-institutional laundry operations. Directions on the label provided the following information regarding preparation and use of the product as a laundry sanitizer: Using the appropriate Ecolab dispenser, inject the product at a rate of 3 ounces per 100 pounds dry laundry in the final rinse water. Treat the laundry for a minimum of 5 minutes at 90–140 °F.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Laundry Sanitizer – For Use During Commercial-Industrial-Institutional Laundry Operations

The effectiveness of laundry sanitizers must be supported by data that show that the product will substantially reduce the numbers of test bacteria on fabric and in laundry water. Laundry additives may either be used as soaking treatments prior to laundering or as treatments added during laundry operations. The label must specify the type of use. Laundry additives may be recommended for household/coin-operated machine use or commercial-industrial-institutional use. The label must specify the type of use. There is a significant difference in the water to fabric ratio between these two uses, which may affect the efficacy of the product. Tests should be conducted using a simulated-use procedure such as Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives" or a simulated use study involving washing machines. Tests should be performed with each of 3 product samples, representing 3 different batches, one of which is at least 60 days old. Tests should be conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Products labeled as being suitable for hospital use must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). Each batch must be tested with 3 fabrics swatches against each of the test organisms. The method employed must include subculturing of both the fabric and the laundry water. The laundry water to media volume ratio must not exceed 1:40. Testing of a 0.5 mL sample of laundry water from the simulated washing device (or a 5 mL sample from the automatic washer) is recommended. Results from a quantitative bacteriological assay must be reported. Results must show a bacterial reduction of 99.9% over

the control count for both fabric and laundry water for each organism tested. The label directions for use of laundry additives should specify the machine cycle in which the product is to be added, as well as water level, temperature, and treatment time. Compatibility of the treatment with other laundry additives should be determined in testing and addressed in labeling, when applicable. These Agency standards are presented in DIS/TSS-13, and do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetrienes, or trichloro-s-triazinetriene.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 459159-01 "Advacare Standard Test Method for Evaluation of Laundry Sanitizers," by Andrea J. Mesaros. Study conducted at AppTec ATS. Study completion date – March 21, 2003. Project Number A01168.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 4352), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. C062821, C081921, and JSH100302) of the product, AdvaCare 120 Sanitizer/Sour, were tested using AppTec Protocol No. ECO01090402.LSAN (not provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." The laboratory report did not indicate which, if any, of the product lots were at least 60 days old at the time of testing. A solution of the product was prepared by diluting ≤ 0.40 mL (0.40, 0.39, and 0.37 mL, respectively) of the product with sterile deionized water to make a total volume of 1 liter. A second set of product lots was prepared in the same way 15 days later. A sterile Nalgene jar was filled with 75 mL of the prepared use solution. The carriers for this test were prepared by boiling 300 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5 grams of Na_2CO_3 , 1.5 grams of Triton X-100 and 3 liters of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 5 minutes and then rinsed in cold water for 5 minutes. After air-drying, the fabric was cut into 5 cm (2 inch) wide strips weighing 15 ± 1 grams. Each strip was wrapped around a spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclaved at 121°C for 20 minutes, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 0.02 mL of the prepared organism culture, and dried in an incubator at $35\text{--}37^\circ\text{C}$ for 30 minutes. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. (The number of swatches per spindle was not specified, but results for three swatches per treatment were reported.) The spindles were placed in the Nalgene jars containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM at 60°C (140°F) for 5 minutes. A 1.0 mL aliquot of the wash water was transferred to a vessel containing 9 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were then vortex mixed to extract fabric-bound microorganisms. The neutralizing medium was then serially diluted by transferring 1.0 mL of the subculture to 9.0 mL of Butterfield's Buffer; representing the 10^{-1} dilution, and continuing in like manner through the 10^{-4} dilution. Each dilution was plated in duplicate tryptic soy agar with 5% sheep blood in aliquots of 1.0 mL. All subcultures were incubated 48 ± 4 hours at $35\text{--}37^\circ\text{C}$. The second set of product dilutions was stored at $2\text{--}8^\circ\text{C}$ for 3 days prior to examination. Following incubation or incubation and storage, the subcultures were then observed for the presence or absence of visible growth. Controls included purity,

sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 459159-02 "Advacare Standard Test Method for Evaluation of Laundry Sanitizers," by Andrea J. Mesaros. Study conducted at AppTec ATS. Study completion date – March 21, 2003. Project Number A01179.

This study was conducted against *Staphylococcus aureus* MRSA (ATCC 33592). Two lots (Lot Nos. C062821 and C081921) of the product, Advacare 120 Sanitizer/Sour, were tested using AppTec Protocol No. ECO01090402.LSAN (not provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." A use solution of the product was prepared by diluting ≤ 0.40 mL (0.40 and 0.39 mL, respectively) of the product with sterile deionized water to make a total volume of 1 liter. A sterile Nalgene jar was filled with 75 mL of the prepared use solution. The carriers for this test were prepared by boiling 300 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5 grams of Na_2CO_3 , 1.5 grams of Triton X-100 and 3 liters of deionized water for 60 minutes. The fabric was rinsed in boiling water for 5 minutes and then rinsed in cold water for 5 minutes. After air-drying, the fabric was cut into 5 cm (2 inch) wide strips weighing 15 ± 1 grams. Each strip was wrapped around a spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclaved at 121°C for 20 minutes, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 0.02 mL of the prepared organism culture, and dried in an incubator at $35\text{--}37^\circ\text{C}$ for 30 minutes. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three swatches. The spindles were placed in the Nalgene jars containing the product and subjected to a simulated tumble-wash at 45-60 RPM at 60°C (140°F) for 5 minutes. A 1.0 mL aliquot of the wash water was transferred to a vessel containing 9 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were then vortex mixed to extract fabric-bound microorganisms. The neutralizing medium was then serially diluted by transferring 1.0 mL of the subculture to 9.0 mL of Butterfield's Buffer; representing the 10^{-1} dilution, and continuing in like manner through the 10^{-4} dilution. Each dilution was plated in duplicate Tryptic Soy Agar with 5% sheep blood. All subcultures were incubated 48 ± 4 hours at $35\text{--}37^\circ\text{C}$. The subcultures were stored at $2\text{--}8^\circ\text{C}$ for 2 days prior to examination. Following incubation and storage, the subcultures were then observed for the presence or absence of visible growth. Controls included purity, sterility, viability, initial inoculum confirmation, carrier population, antibiotic resistance, and neutralization confirmation.

Note: An antibiotic resistance profile for the test organism was provided in the laboratory report. The laboratory verified that the test organism was resistant by performing the Kirby Bauer Susceptibility assay.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V RESULTS

MRID Number 459159-01

Organism	Lot No.	Average No. Surviving (CFU/ swatch)	Microbes Initially Present (mean CFU/ swatch)	"Wash" Water Test Results (CFU @ 10 ⁻⁶)	"Wash" Water Control (CFU/ mL)	% Red.
<i>Klebsiella pneumoniae</i>	C062821	<27	3.6 x 10 ⁴	0, 0	1.0 x 10 ³	>99.9
	C081921	<10		0, 0		>99.9
	JSH100302	<10		0, 0		>99.9
Test date: 12/18/02						
<i>Pseudomonas aeruginosa</i>	C062821	<10	8.2 x 10 ⁵	0, 0	4.9 x 10 ⁴	>99.9
	C081921	<10		0, 0		>99.9
	JSH100302	<10		0, 0		>99.9
Test date: 12/18/02						
<i>Staphylococcus aureus</i>	C062821	<10	5.2 x 10 ⁶	0, 0	1.33 x 10 ⁵	>99.9
	C081921	<10		0, 0		>99.9
	JSH100302	<10		0, 0		>99.9
Test date: 12/03/02						

MRID Number 459159-02

Organism	Lot No.	Average No. Surviving (CFU/ swatch)	Microbes Initially Present (mean CFU/ swatch)	"Wash" Water Test Results (CFU @ 10 ⁻⁶)	"Wash" Water Control (CFU/ mL)	% Red.
<i>Staphylococcus aureus</i> MRSA	C062821	<10	3.7 x 10 ⁵	0, 0	1.12 x 10 ⁵	>99.9
	C081921	<10		0, 0		>99.9
Test date: 12/18/02						

VI CONCLUSIONS

The submitted efficacy data (MRID No. 459159-01) does not support the use of the product, AdvaCare 120 Sanitizer/Sour, as a laundry additive for sanitizing laundry during commercial-industrial-institutional laundry operations against *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 4352), and *Pseudomonas aeruginosa* (ATCC 15442) for a contact time of 5 minutes at 140°F because none of the batches tested were over 60 days old and because of incorrect rate application. The label states that the rate of application is 3 ounces product/100 pounds of dry laundry which is equal to 0.029 mL product/15 gram swatch. Because the rate of application is incorrect, all calculations derived from the rate calculation are incorrect also.

The submitted efficacy data (MRID No. 459159-02) does not support the use of the product, AdvaCare 120 Sanitizer/Sour, as a laundry additive for sanitizing laundry during commercial-industrial-institutional laundry operations against *Staphylococcus aureus* MRSA (ATCC 33592) for a contact time of 5 minutes at 140°F because none of incorrect rate application. The label states that the rate of application is 3 ounces product/100 pounds of dry laundry which is equal to 0.029 mL product/15 gram swatch. Because the rate of application is incorrect, all calculations derived from the rate calculation are incorrect also.

VII RECOMMENDATIONS

1. The registrant needs to conduct new studies against *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 15442), and *Staphylococcus aureus* (MRSA) because use dilutions were prepared. Because the label does not state the amount of water that is appropriate for use, the Agency is unsure that the use dilution is appropriate. The study only states that about 40 mL of product was diluted until a volume of 1 L was reached. The label only states the application rate of the product is 3 ounces/100 pounds dry laundry. In the new study conducted against *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 4352), and *Pseudomonas aeruginosa* (ATCC 15442), the dates of the batches must be included as one of the batches used in the study must be over 60 days old.
2. The registrant should include the protocol that was used to conduct the study.

VIII LABELING COMMENTS

1. The label must include the amount of water that is appropriate for use, the Agency is unsure that the use dilution is appropriate. For example, the label must state that the application rate is 3 ounces/100 pounds dry laundry per 200 gallons of water.
2. The proposed label and the last accepted label have several typographical errors.
 - a. on page 1 under FIRST AID, "Take of contaminated clothing" should be "Take off contaminated clothing."

- b. on page 1 under FIRST AID, "Do not anything by mouth" should be "Do not give anything by mouth."
- c. on page 1 under ENVIRONMENTAL HAZARDS, "(NPDES) permit and permitting authority" should be "(NPDES) permit and the permitting authority."
- d. on page 2 under STORAGE & DISPOSAL, "or puncture and dispose of in sanitary landfill, or incineration" should be "or puncture and dispose of in a sanitary landfill, or by incineration."
- e. on page 2 under STORAGE & DISPOSAL, "Dispose of empty container in a sanitary landfill, . . ., or is allowed by burning" should be "Dispose of empty container in a sanitary landfill, . . ., or if allowed by state and local authorities, by burning."